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The differences in chromogranin A (CgA) concentrations measured in serum and in plasma by IRMA and ELISA methods

Różnice w stężeniu chromograniny A (CgA) badanej w surowicy i w osoczu metodami IRMA i ELISA

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Abstract

Introduction: Chromogranin A (CgA) is regarded as a major, nonspecific marker of neuroendocrine tumors (NET). Its estimation appears helpful for diagnostic purposes and is particularly useful for monitoring the treatment of NET. It must be kept in mind, however, that various factors, drugs, or coexisting diseases may influence the outcome of CgA measurement in blood. One such analytical factor is the sort of studied biological material, whether it is plasma or serum.
The aim of our study was to compare directly the results of CgA concentrations measured in serum and in plasma by IRMA and ELISA.
Material and methods: We analysed 122 samples of EDTA-plasma and 122 samples of serum by IRMA method, 20 samples of EDTA-plasma and 20 samples of serum by IRMA and ELISA, 25 heparinised-plasma samples and 25 samples of EDTA-plasma by IRMA and ELISA methods, and 8 EDTA-plasma, heparinised-plasma, and serum samples by IRMA and ELISA.
The material for comparative study was obtained during the same blood collection from the same subjects (volunteers and patients with NET). CgA was measured with the use of kits manufactured by CIS bio International (France).
Results: CgA concentrations were markedly higher in plasma than in serum. Using the IRMA method, the difference in the CgA range between 10–100 ng/mL approached 20–70% (median 61 *v.* 42), in the range 101–300 ng/mL —– 12–60% (median 147 *v.* 101), and in the CgA range 301–1076 ng/mL — 14–40% (median 486 *v.* 356). The differences between results in serum and plasma using ELISA were similar but slightly smaller. There was no significant difference between CgA levels in EDTA and heparinised-plasma samples, and the results of measurements performed by IRMA and ELISA in most cases were similar.
Conclusions: Referring each individual CgA result to the proposed reference range (or cut-off value) we must take into account whether the measurement is performed in plasma or in serum. **(Pol J Endocrinol 2010; 61 (4): 346–350)**

Key words: *chromogranin A, CgA, CgA-IRMA, CgA-ELISA, neuroendocrine tumours*

Streszczenie

Wstęp: Chromogranina A (CgA, *chromagranin A*) jest głównym, niespecyficznym markerem guzów neuroendokrynnych (NET, *neuroendocrine tumors*). Oznaczanie jej stężenia jest pomocne w diagnostyce biochemicznej oraz szczególnie użyteczne w monitorowaniu efektów leczenia guzów NET. Istnieje wiele czynników analitycznych, leków i współistniejących chorób mogących wpływać na stężenie CgA we krwi. Jednym z takich czynników może być rodzaj użytego materiału biologicznego do badania, to znaczy czy jest to osocze czy surowica. Celem pracy było porównanie wyników oznaczenia CgA badanej w surowicy i w osoczu metodą IRMA i ELISA.
Materiał i metody: Do badań porównawczych użyto 122 próbek osocza (EDTA) i 122 próbek surowicy, w których stężenie CgA oznaczono metodą IRMA, 20 próbek osocza EDTA i 20 próbek surowicy — metodami IRMA i ELISA, 25 próbek osocza EDTA i 25 próbek osocza heparynowego — metodami IRMA i ELISA oraz 8 próbek osocza EDTA, osocza heparynowego i surowicy metodami IRMA i ELISA. Materiał do porównania uzyskano z jednoczasowego pobrania krwi od tych samych osób (ochotników i pacjentów z NET).
Stężenie CgA oznaczono zestawami odczynników firmy CIS bio International (Francja).
Wyniki: Stężenie CgA w osoczu było istotnie wyższe niż w surowicy. Różnice te w przedziale stężeń 10–100 ng/ml wyniosły 20–70% (mediana 61 *v.* 42), w zakresie 101–300 ng/ml wyniosły 12–60% (mediana 147 *v.* 101), natomiast przy stężeniach 301–1076 ng/ml wyniosły 14–40% (mediana 486 *v.* 356). Stężenia CgA mierzone w tych samych próbkach metodami IRMA i ELISA były zbliżone, aczkolwiek różnice między oznaczeniami w surowicy i w osoczu były trochę mniejsze w metodzie ELISA. Nie stwierdziliśmy natomiast istotnych różnic między stężeniami CgA badanego w osoczu heparynowym i w osoczu EDTA.
Wnioski: Odnosząc każdy indywidualny wynik CgA do podanego zakresu referencyjnego (albo punktu odcięcia), musimy mieć na uwadze to, czy materiałem do badania było osocze czy surowica. **(Endokrynol Pol 2010; 61 (4): 346–350)**

Słowa kluczowe: *chromogranina A, CgA, CgA-IRMA, CgA-ELISA, guzy neuroendokrynne*

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Introduction

Chromogranin A (CgA) is a 49-kDa acidic glycoprotein [1]. CgA is physiologically released by exocytosis, and can be detected in blood [2, 3].

The first immunoassay for detection of circulating CgA levels in blood was a RIA method developed in 1984 by O'Connor and Bernstein. In 1989, Dillen et al. developed an EIA method with enzyme-conjugated CgA. Further works applied sandwich methods using two monoclonal or polyclonal antibodies [4–7].

Chromogranin A (CgA) is regarded as a major, non-specific marker of neuroendocrine tumours (NET). Its estimation appears helpful for diagnostic purposes and is particularly useful for monitoring the treatment of NET [8–11]. It must be kept in mind, however, that various factors, drugs, or coexisting diseases may influence the outcome of CgA measurement in blood [12]. One such analytical factor is the sort of studied biological material, whether it is plasma or serum [13].

The aim of our study was to compare the results of CgA measurements in serum, plasma-EDTA, and plasma-heparin using commercial IRMA and ELISA methods.

Material and methods

The materials for our study were serum, plasma-EDTA, and plasma-heparin samples obtained from the same blood collections in healthy volunteers and patients diagnosed for the possible presence of NET or being monitored during specific treatment for GEP-NET, carcinoid, pheochromocytoma, adrenal carcinomas, or MEN syndromes.

Venous blood samples were drawn into tubes containing EDTA, heparin, or clot activator, centrifuged at 4°C (1500–2000 g) for 15 minutes, then plasma and serum were separated and stored at –20°C until assayed.

We analysed 122 samples of EDTA-plasma and 122 samples of serum by IRMA method, 20 samples of EDTA-plasma and 20 samples of serum by IRMA and ELISA methods, 25 heparinised plasma samples and 25 samples of EDTA-plasma by IRMA and ELISA methods, and 8 EDTA-plasma, heparinised-plasma, and serum samples by IRMA and ELISA methods.

CgA was measured with the use of kits manufactured by CIS bio International (France). In both methods a recombinant human CgA was used as standard, and the applied monoclonal antibodies recognized the central domain of the molecule aa. 145–245 (intact CgA), and probably only some of the circulating CgA fragments. The detection limit of IRMA is 1.5 ng/mL, and that of ELISA is 2.6 ng/mL. The reference value for the IRMA method proposed by the manufacturer (calculated on a group of 162 volunteers) in serum samples is 19.4–98.1 ng/ml (median 41.6) and for plasma-EDTA (calculated on a group of 50 volunteers) it is supposed to be in the range 20–150 ng/mL (median 56) (those latter data were obtained from the CIS website). The reference value for the ELISA CIS method, as determined only for serum samples on a group of 114 healthy subjects, was 27–94 ng/mL (median 44).

The study was approved by the Ethical Committee at the Medical Centre for Postgraduate Education.

All results are expressed as median and arithmetic mean ± SD. Differences between the groups are presented as percentages.

Results

Table I presents the results of CgA concentrations as measured by IRMA in plasma-EDTA and serum samples of 122 patients in 3 ranges of concentrations. In the range 10–100 ng/mL, the difference between plasma-EDTA and serum was 20–70% (median 61 *v.* 42), in the

Table I. ***The differences in chromogranin A (CgA) concentrations measured in plasma-EDTA and serum by IRMA method in three concentration ranges***

Tabela I. ***Różnice w stężeniu chromograniny (CgA) oznaczanej w osoczu EDTA i surowicy metodą IRMA w trzech zakresach stężeń***

	n= 122	Plasma-EDTA	Serum	Difference (%)	
10–100 ng/mL	83	x = 73 ng/mL SD = 48 median = 61	x = 56 ng/mL SD = 42 median = 42	20–70% (x = 46%)	plasma-EDTA >> serum
101–300 ng/mL	28	x = 157 ng/mL SD = 127 median = 147	x = 117 ng/mL SD = 128 median = 101	12–60% (x = 36%)	plasma-EDTA >> serum
301–1076 ng/mL	11	x = 541 ng/mL SD = 233 median = 486	x = 416 ng/mL SD = 203 median = 356	14–40% (x = 31%)	plasma-EDTA > serum

Table II. *The differences in chromogranin A (CgA) concentrations measured in plasma-EDTA and serum by IRMA and ELISA methods*

Tabela II. *Różnice w stężeniu chromograniny A (CgA) oznaczanej w osoczu EDTA i surowicy metodą IRMA i ELISA*

IRMA		ELISA	
Plasma-EDTA n = 20	**Serum n = 20**	**Plasma-EDTA n = 20**	**Serum n = 20**
x = 329 ng/mL SD = 247 median = 249	x = 254 ng/mL SD = 205 median = 184	x = 353 ng/mL SD = 261 median = 251	x = 308 ng/mL SD = 243 median = 222
12–48% plasma > serum		4–33% plasma > serum	

Table III. *The differences in chromogranin A (CgA) concentrations measured in plasma-EDTA and plasma-heparin by IRMA and ELISA methods*

Tabela III. *Różnice w stężeniu chromograniny A (CgA) oznaczanej w osoczu EDTA i osoczu heparynowym metodą IRMA i ELISA*

IRMA		ELISA	
Plasma-EDTA n = 25	**Plasma-heparin n = 25**	**Plasma-EDTA n = 25**	**Plasma-heparin n = 25**
x = 158 ng/mL SD = 192 median = 87	x = 161 ng/mL SD = 196 median = 88	x = 140 ng/mL SD = 193 median = 76	x = 145 ng/mL SD = 203 median = 83
1–8% plasma-EDTA = plasma-heparin		1–14%* plasma-EDTA ≈ plasma-heparin	

*in a few cases the difference amounted 12–34%

range 101–300 ng/mL, the difference was 12–60% (median 147 *v.* 101) and in the range 301–1076 ng/mL it was 14–40% (median 486 *v.* 356).

Table II presents the results of CgA concentrations as measured by both IRMA and ELISA methods in the other 20 patients. The concentration of CgA appeared higher in plasma-EDTA than serum, and the difference was 12–48% (median 249 *v.* 184) for the IRMA method, and 4 –33% (median 251 *v.* 222) for ELISA.

The Table III shows the CgA results in plasma-EDTA and plasma-heparin samples of 25 patients as measured by IRMA and ELISA methods. By IRMA method, the median CgA concentrations for plasma-EDTA and plasma-heparin were 87 *v.* 88 (the difference was between 1–8%). By ELISA method, the median concentrations were 76 *v.* 83 only in 6 of 25 cases (only in 6 of 25 cases this difference was 12–34%).

In the group of healthy volunteers (Table IV), the median concentrations of CgA in plasma-EDTA and plasma-heparin for IRMA method were 49 *v.* 47, and for the ELISA methods, the median was 35 *v.* 41, whereas the differences between CgA concentrations in plasma-EDTA and serum samples were 28–76% (median 49 *v.* 34) for IRMA and 22–68% (median 35 *v.* 19) for ELISA, respectively.

Discussion

Currently, blood CgA levels can be measured using three methods: IRMA, ELISA, and RIA. Comparing the CgA concentration in serum and plasma, obtained at the same time from the same subjects, we observed higher levels in plasma than in serum, regardless of the analytical method used (IRMA or ELISA of the same producer).

In general, all commercially available CgA kits are characterized by relatively high specificity for CgA molecules (intact) but may differ slightly in the matter of recognition of the CgA fragments or aggregates.

The questions arise:

1. Should current analytical tests recognize mainly intact CgA or both the intact sequence and major fragments of CgA?
2. What is the cause of the observed differences in the concentrations of CgA tested in plasma and serum?
3. Could it be due to the different stability of the CgA in the environment of plasma and serum?

Table IV. *The chromogranin A (CgA) concentrations measured in plasma-EDTA, plasma-heparin, and serum in a healthy volunteer group by IRMA and ELISA methods*

Tabela IV. *Stężenie chromograniny A (CgA) oznaczane w osoczu-EDTA, osoczu heparynowym i surowicy w grupie zdrowych ochotników metodą IRMA i ELISA*

IRMA			ELISA		
Plasma-EDTA	**Plasma-heparin**	**Serum**	**Plasma-EDTA**	**Plasma-heparin**	**Serum**
n = 8	**n = 8**	**n = 8**	**n = 8**	**n = 8**	**n = 8**
x = 46 ng/mL SD = 17 median = 49	x = 43 ng/mL SD = 15 median = 47	x = 33 ng/mL SD = 13 median = 34	x = 38 ng/mL SD = 13 median = 35	x = 42 ng/mL SD = 12 median = 41	x = 27 ng/mL SD = 14 median = 19
	2–6% 28–76%			3–9%* 22–68%	

*in two cases the difference amounted to 25%

Chromogranin A is a calcium-binding protein and can aggregate at high concentrations of free Ca^{2+} ions, so the measured CgA blood level could be affected by the presence of such Ca^{2+} ions [14, 15]. Anticoagulation occurs through the binding of Ca^{2+} ions and the inhibition of thrombin action, whereas in venous blood samples drawn into tubes containing clot activator, the calcium ions remain free. We suppose that the possible reason for the detected differences could be a partial aggregation of CgA in the environment of free calcium ions, which can be prevented by the presence in the samples of EDTA or heparin. Another possible explanation for the noted differences is that some small CgA fragments might be bound by the particles of a clot activator used in serum samples.

Modlin IM et al. (2010) mention in their paper that CgA measurements in serum and plasma are concordant [16]; however, from the work of Woltering EA et al. (2006) [17,18] (from Fig. 1) cited by them in that matter, it appears that although correlation is high, the CgA levels in plasma are c. 50% higher than in serum.

Our study showed that the difference between CgA plasma and serum levels amounted to 20–70% (higher levels were observed in plasma samples), whereas in general there was no significant difference in the CgA concentrations measured in EDTA and the heparin plasma environment.

Another important matter is an appropriate cut-off value, which to some extent should be adjusted to the sort of biological material used (whether it is serum or plasma). The producer of the assay kits, CIS bio, set the upper value for serum at 98 ng/mL for IRMA and 94 ng/mL for ELISA, whereas for plasma-EDTA it is set at 150 ng/mL (from website information). So the cut off value for plasma CgA is about 50% higher than for the serum samples.

Other authors accepted a cut-off value for the serum similar to that specified by the kit manufacturer. Leon A et al. (2005) [19] determined the cut-off value to be 87 ng/mL for the IRMA method, whereas the Italian group of authors (2007) [20] proposed a cut-off value of 53 ng/mL for the same IRMA method despite the fact that in 95% of their control subjects the upper limit was 86 ng/mL. Both of the above-mentioned authors measured the concentrations of CgA only in serum. However, Bìlek R et al. (2008) [21], who measured the concentration of CgA in EDTA-plasma of patients with pheochromocytoma by ELISA, adopted the value of the cut-off level at 130 ng/mL. This value is also c. 50% higher than that proposed by Leon A et al. for the serum samples and lower than that suggested by the CIS manufacturer for plasma samples. This matter needs further evaluation on larger groups of patients and healthy subjects.

At this time, the question arises whether we should perform measurements of CgA only either in plasma-EDTA or in heparinised-plasma, or the serum samples are good as well. We think that the results of the CgA determination in serum, although lower than in plasma, are clinically useful too, provided the appropriate reference range is adopted (especially cut-off value), and the numerous other causes of elevated CgA levels in blood are considered.

Conclusions

1. We found significant differences in the concentrations of CgA measured in plasma and in serum, both by IRMA and ELISA methods.
2. No marked differences in the determinations of CgA concentration in EDTA-plasma and heparinised-plasma were observed.

3. To have valid and comparable results, it is obligatory to measure CgA concentrations in the same sort of material using the same method, preferably in the same laboratory, and taking into account various factors potentially affecting its concentration in blood.

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